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(54) Amplification of long nucleic acid sequences by PCR

(57) Methods and reagents are provided for the amplification of nucleic acid sequences, e.g. DNA sequences, longer than 10 kilobases by the polymerase chain reaction (PCR). The methods use compositions consisting of a primary thermostable DNA polymerase from *Thermus thermophilus* combined with a lesser amount of a secondary thermostable DNA polymerase possessing a 3'-to-5' exonuclease activity from *Thermococcus litoralis*, *Pyrococcus* species GB-D or *Thermotoga maritima*. The DNA polymerase compositions, when used with the disclosed reaction buffer, enable amplifications of DNA sequences up to at least 42.2 kilobases in length.

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EUROPEAN SEARCH REPORT

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EP 95 10 2141

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	US-A-5 001 050 (L. BLANCO ET AL.) * column 11, line 20 - line 45 * * column 4, line 13 - line 57 * * column 2, line 60 - column 3, line 12 *	1	C12Q1/68 C12Q1/48 C12N9/12
A	* column 2, line 8 - line 28 * ---	3,4	
Y	PCR METHODS & APPLICATIONS, vol. 3, no. 2, February 1993 COLD SPRING HARBOR US, pages 115-119, L.D. OHLER ET AL. 'Use of a sensitive fluorescent intercalating dye to detect PCR products of low copy number and high molecular weight.' * page 119, column 1, line 17 - line 23 *	1	
Y	THE NEB TRANSCRIPT, vol. 3, no. 1, March 1991 BEVERLY US, page 4 XP 000391405 'Recombinant Vent DNA polymerase' * paragraph 2 *	1	
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Y	NUCLEIC ACIDS RESEARCH, vol. 18, no. 4, 1990 OXFORD GB, pages 999-1005, S KWOK ET AL. 'Effects of primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies' * the whole document *	1	C12Q C12N
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The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 19 December 1995	Examiner De Kok, A
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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A	C.R. NEWTON ET AL. (EDS.) 'PCR' 1994, BIOS SCIENTIFIC PUBLISHERS, OXFORD GB * page 12 - page 16 *	1,2	
A	SCIENCE, vol. 252, no. 5013, 21 June 1991 pages 1643-1651, XP 000381755 H.A. ERLICH ET AL. 'RECENT ADVANCES IN THE POLYMERASE CHAIN REACTION' * the whole document *	1-9	
D,Y	NUCLEIC ACIDS RESEARCH, vol. 20, no. 3, 11 February 1992 OXFORD GB, page 623 M.R. PONCE ET AL. 'PCR amplification of long DNA fragments' * the whole document *	5	
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P,X	SCIENCE, vol. 263, no. 5153, 18 March 1994 WASHINGTON US, pages 1564-1565, J COHEN 'Long PCR leaps into larger DNA sequences' * the whole document *	1-9	
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Place of search BERLIN		Date of completion of the search 19 December 1995	Examiner De Kok, A
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